ORIGINAL ARTICLE



Surveillance for enteric pathogens in a case-control study of acute diarrhea in Western Kenya

Brett E. Swierczewski^{a,*}, Elizabeth A. Odundo^a, Margaret C. Koech^a, Janet N. Ndonye^a, Ronald K. Kirera^a, Cliff P. Odhiambo^a, Erick K. Cheruiyot^a, Max T. Wu^b, James E. Lee^b, Chunlin Zhang^b and Edwin V. Oaks^b

^aUnited States Army Medical Research Unit - Kenya, Kericho Field Station, PO Box 1357, Hospital Road, Kericho, Kenya 20220; ^bDivision of Bacterial and Rickettsial Diseases, Walter Reed Army Institute of Research, Silver Spring, MD, USA

*Corresponding author: Tel: +1 301 310 7574; Fax: +1 301 319 9801; E-mail: brett.swierczewski@us.army.mil

Received 3 May 2012; revised 24 September 2012; accepted 13 November 2012

Background: Acute diarrhea remains a major public health problem in East African nations such as Kenya. Surveillance for a broad range of enteric pathogens is necessary to accurately predict the frequency of pathogens and potential changes in antibiotic resistance patterns.

Method: Stool samples were collected from September 2009 to September 2011; 193 and 239 samples, from age-matched cases and asymptomatic controls, were collected, respectively, from Kericho and Kisumu District Hospitals in western Kenya. Bacterial pathogens were identified by conventional microbiological methods; antibiotic susceptibility of bacterial isolates was ascertained using the MicroScan WalkAway 40 Plus. An enzyme immunoassay kit was used to detect rotavirus, and ova and parasite examination was conducted by microscopy and an enzyme immunoassay.

Results: Rotavirus (10.2% and 10.5%) and *Shigella* (11% and 8%) were isolated significantly more often in the cases than the controls from Kericho and Kisumu District Hospitals respectively. The diarrheagenic *Escherichia coli, Campylobacter jejuni* and *Salmonella* were found most often in the cases while *Giardia lamblia* and *Entamoeba histolytica/E. dispar* were found more often in the controls. Most pathogens were isolated from children under 5 years old. More than 50% of the *Shigella, Salmonella* and diarrheagenic *E. coli* isolates were multidrug resistant to ampicillin, tetracycline and trimethoprim/sulfamethoxazole with several enteroaggregative and enterotoxigenic *E. coli* isolates producing extended-spectrum beta-lactamases.

Conclusion: Accurate epidemiologic information on acute diarrheal illness in Kenya will be critical for augmenting existing diarrhea management policies in terms of treatment and to strengthen future community awareness and health promotion programs.

Keywords: Gastroenteritis, Protozoa, Rotavirus, Shigella, Escherichia coli, Antimicrobial resistance

Introduction

Diarrheal disease continues to be a major cause of morbidity and mortality worldwide, particularly in areas of poor sanitation and hygiene. Acute diarrheal disease accounts for more than 1.8 million deaths annually, particularly in children under 5 years of age, and more than 40% of those deaths occur in Africa. Globally, the most frequently identified pathogens of acute diarrhea are bacteria (diarrheagenic *Escherichia coli, Vibrio* spp., *Salmonella, Shigella*, and *Campylobacter*), viruses (rotaviruses and noroviruses) and parasites (*Entamoeba histolytica, Giardia lamblia*, and *Cryptospororidium parvum*). The prevalence of these etiologic agents for diarrhea in any particular region is likely dependent on several factors, including sanitation infrastructure, social culture and personal hygiene practices.

Enteric surveillance studies conducted in Kenya over the past 20 years have been largely focused on certain bacterial causes, with a few individual studies reporting on parasitic and viral causes of diarrhea. Additionally, a few enteric pathogen prevalence studies have been conducted in specific geographic regions of Kenya. In many parts of Kenya, the laboratory capability to detect a broad spectrum of pathogenic agents is lacking; as a result there are insufficient data on the specific pathogens causing acute diarrhea in Kenya, particularly in rural areas. Empirical diagnosis of an enteropathogen by a healthcare provider without laboratory confirmation can result in missed diagnosis or the prescription of inappropriate treatment. To address this lack of data, a case-control acute diarrhea study was begun in September 2009 to determine the prevalence of enteric pathogens in patients with acute diarrhea.

maintaining the data needed, and c including suggestions for reducing	lection of information is estimated to ompleting and reviewing the collect this burden, to Washington Headqu uld be aware that notwithstanding an DMB control number.	ion of information. Send comments arters Services, Directorate for Info	s regarding this burden estimate ormation Operations and Reports	or any other aspect of the s, 1215 Jefferson Davis	nis collection of information, Highway, Suite 1204, Arlington	
1. REPORT DATE DEC 2012		2. REPORT TYPE		3. DATES COVE 00-00-2012	red 2 to 00-00-2012	
4. TITLE AND SUBTITLE				5a. CONTRACT	NUMBER	
	teric pathogens in a	case-control study	of acute	5b. GRANT NUM	ИBER	
diarrhea in Wester	п кенуа			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)				5d. PROJECT NU	JMBER	
				5e. TASK NUMBER		
				5f. WORK UNIT NUMBER		
Walter Reed Army	ZATION NAME(S) AND AD Institute of Research S,Silver Spring,MD		erial and	8. PERFORMING REPORT NUMB	G ORGANIZATION ER	
9. SPONSORING/MONITO	RING AGENCY NAME(S) A	ND ADDRESS(ES)		10. SPONSOR/M	ONITOR'S ACRONYM(S)	
				11. SPONSOR/M NUMBER(S)	ONITOR'S REPORT	
12. DISTRIBUTION/AVAII Approved for publ	ABILITY STATEMENT ic release; distributi	on unlimited				
13. SUPPLEMENTARY NO	OTES					
14. ABSTRACT						
15. SUBJECT TERMS						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON	
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified	Same as Report (SAR)	8		

Report Documentation Page

Form Approved OMB No. 0704-0188 Stool samples were collected from patients seen in an outpatient setting in two district hospitals in western Kenya and processed for enteric pathogens at the microbiology laboratory of the US Army Medical Research Unit-Kenya, located in Kericho, Kenya. Here we report the enteric pathogen distribution and the antibiotic resistance patterns after 2 years of surveillance.

Materials and methods

Study sites

Stool samples were collected from September 2009 to September 2011 at Kericho District Hospital and Kisumu District Hospital. Kericho District Hospital is located in Kericho (lat 00°22′S, long 35°15′), in the South Rift Valley of Kenya. Kericho is a major area for tea production in Kenya, because of its high altitude and temperate climate. Kisumu District Hospital is in the sprawling city of Kisumu. Kisumu (lat 00°03′S, long 34°45′E, in Nyanza Province, is adjacent to Lake Victoria and is the third largest city in Kenya after Nairobi and Mombasa. Both facilities are public hospitals operated by the Kenya Ministry of Health and provide services to the local and outlying communities respectively.

Enrollment and specimen and data collection

Acute diarrhea was defined as three or more episodes of loose, bloody or watery stool in less than a 24-h period, but with these symptoms not lasting more than 14 days. Age-matched controls (reported for medical reasons other than acute diarrhea) were recruited from the same hospital in which the patients were seen and had no episodes of diarrhea within the weeks prior to enrollment. All patients and controls were recruited from the outpatient population only. Informed consent was obtained directly from study participants aged 18 years and over, and from a parent or quardian of those below that age. After stool collection, demographic and historical clinical details were captured on questionnaires for both patients and controls; data collected included place of residence, age, sex, type of water source used daily (municipal, well, borehole and rainwater) and any overt clinical symptoms. A detailed physical examination was also conducted by a clinical officer at the time of enrollment to determine the study participants' nutritional status.

Specimen collection

A portion of the fresh stool specimen was aliquoted into Cary-Blair transport medium (Medical Chemical Corporation, Torrance, CA, USA), 10% formalin and a clean vial which was stored at -20°C at the site. All vials were transported in cool boxes to the microbiology laboratory in Kericho for processing.

Stool processing and analysis

Stool preserved in Carey-Blair transport medium was inoculated onto MacConkey agar (MAC), sheep blood agar (BAP), MacConkey-sorbitol agar (SMAC), Hektoen enteric agar (HE), cefsulodin-irgasan-novobiocin agar (CIN), cefoperazone-vancomycin-amphotericin agar (CVA), and thiosulfate-citrate-bile-sucrose agar (TCBS).

Plates except for CVA were incubated overnight at 37 °C for 18-24 h. CVA plates were incubated at 42 °C for 48 h in a microaerophilic environment in a Campy pouch (Becton Dickinson, Franklin Lakes, NJ, USA). Subculture plates were inoculated and incubated as described above. Single colonies exhibiting the proper characteristics on the various media described above (except for Campylobacter spp. identification) were processed by the MicroScan WalkAway 40 automated bacterial identification and antibiotic susceptibility platform (Siemens, Erlangen, Germany). Briefly, cell suspensions of isolates at an optical density of 0.5 were inoculated into Microscan Gram-negative conventional (overnight) panels according to the manufacturer's instructions and incubated inside the platform overnight at 37 °C. Identification and antibiotic susceptibility results of the bacterial isolates were available the following day. Suspected Campylobacter isolates were characterized at the species level using confirmatory phenotypic analyses for Campylobacter. 14

A multiplex PCR was used for the detection of enterotoxigenic E. coli (ETEC) and enteroaggregative E. coli (EAEC) according to previously described methods. Five lactose-fermenting colonies of similar morphology were isolated from MAC plates and bacterial DNA was extracted by resuspending the colonies in 50 μ l of sterile water, boiling the suspension for 5 min and centrifuging at 10 000 g for 1.5 min. The resulting supernatant was used as the DNA template for the PCR reaction. To detect ETEC, the following primers sets were used to amplify a 190 bp fragment of the heat-stable toxin and a 450 bp fragment of the heat labile toxin respectively respectively:

5'-ATTTTTCTTTCTGTATTGTCT-3'

5'-CACCCGGTACAAGCAGGATT-3'

5'-GGCGACAGATTATACCGTGC-3'

5'-CGGTCTCTATATTCCCTGTT-3'.

The following primers were used to amplify a 324 bp fragment of the *aaaR* aene for the detection of EAEC:

5'-CTAATTGTACAATCGATGTA-3'

5'-CTGAAGTAATTCTTGAAT-3'.

Cycling conditions were as follows: 95°C for 45 s, 50°C for 45 s, 72°C for 45 s with a final elongation step of 72°C for 10 min. Amplicons were visualized using gel electrophoresis. American Type Culture Collection (ATCC) strains for ETEC and EAEC were used as positive controls in every PCR reaction.

The detection of G. lamblia, E. histolytica/E. dispar, and C. parvum was performed from September 2009 to September 2010, using the Triage Parasite Panel (Biosite Inc., San Diego, CA, USA), according to the manufacturer's instructions. From October 2010 to September 2011, routine ova and parasite examination was conducted via microscopy. A comparison of the two methods was conducted using more than 200 stool samples to ensure there were no major differences between the two methods. 16 For ova and parasite examination, stool was concentrated using the Mini Parasep SF kit (DiaSys, Wokingham, Berkshire, England) according to the manufacturer's instructions. To examine stool samples for ova and parasites, two wet mounts were prepared for each specimen (one stained with iodine) and analyzed by two laboratory technicians. For C. parvum identification, a modified acid-fast stain was used according to previously described methods. 17 For all ova and parasite examinations, control slides for E. histolytica/E. dispar, G. lamblia and C. parvum were prepared and used in real time along with the samples. Rotavirus was detected using the Premier Rotaclone kit (Meridian Bioscience Europe) according to the manufacturer's instructions.

Antibiotic susceptibility testing

Antibiotic susceptibility testing for all bacterial isolates except for *Campylobacter* was conducted using the Microscan automated platform as described above. Each bacterial isolate's sensitivity was tested against 26 antibiotics in specific Gram-negative conventional (overnight) panels. Resistance was determined using standards as defined by the Clinical and Laboratory Standards Institute (CLSI, Wayne, PA, USA). E-tests (Biomerieux, Craponne, France) were used for antibiotic susceptibility testing for *Campylobacter* isolates. Antibiotics used were ampicillin, ciprofloxacin, tetracycline, trimethoprim/sulfamethoxazole and gentamicin. CLSI standards for Enterobacteriaceae were used to interpret results for *Campylobacter* antibiotic susceptibility.

Statistical analysis

Odds ratios and 95% confidence intervals were calculated using multivariate logistic regression for statistical analysis on case-control data. A χ^2 test was used to determine statistical significance of the numbers of enteric organisms in the different age groups. Data were analyzed using GraphPad Prism Version 5.01(GraphPad Software Inc., La Jolla, CA, USA). A p value of <0.05 was considered statistically significant.

Results

A total of 386 stool samples (193 from patients and 193 from controls) was collected from Kericho District Hospital from 25 September 2009 to 30 September 2011. The median ages for patients and controls from Kericho District Hospital were 2 years 5 months and 2 years 9 months respectively; 53% of the patients and 57% of the controls were male. The proportion of patients and controls in specific age groups were as follows: 0−1 year: 38%; 2−5 years: 38%; 6−19 years: 9%; ≥20 years: 15%. Physical examinations by clinical officers found that 2.5% (5/193) of the controls and 11% (21/193) of the patients were malnourished and severely dehydrated. The median age for these individuals was 1 year 6 months. Bloody diarrhea was observed in 6.8% (13/193) of the cases, the mean duration of diarrhea being 48 h.

A total of 478 stool samples (239 from patients and 239 from controls) was collected from Kisumu District Hospital from 27 November 2009 to 30 September 2011. The median ages for cases and controls for Kisumu District Hospital were 1 year 11 months and 2 years respectively; 43% of the cases and 53% of the controls were male. The proportion of cases and controls in specific age groups were as follows: 0−1 year: 49%; 2−5 years: 15%; 6−19 years: 7%; ≥20 years: 29%. Physical examinations by clinical officers found that 0.75% (2/239) of the controls and 4.6% (11/230) of the patients were malnourished and severely dehydrated. The median age for these individuals was 2 years. Bloody diarrhea was observed in 9.6% (23/239) of the patients, the mean duration of diarrhea being 48.5 h.

The most common organisms isolated from patients at Kericho District Hospital were *Shigella* (11%, 21/193), rotavirus

(10.2%, 20/193) and *G. lamblia* (10%, 19/193) (Table 1). Of these, *Shigella* and rotavirus were isolated significantly more often from the patients (11% vs 1%, 10.2% vs 3% respectively) and both were associated with diarrhea (OR = 12.3, OR = 3.6 respectively; p < 0.05). Other organisms isolated more frequently from the patients, though differences were not statistically significant, were *C. jejnui*, ETEC, enterohemorrhagic *E. coli* (EHEC) and *E. histolytica/E. dispar*. Identical numbers of EAEC and *Salmonella* isolates were found in patients and controls, while *C. parvum* was detected more often in the controls (Table 1). Of the 22 *Shigella* isolates detected in the patients, 32% (7/22) were *S. sonnei*, 32% (7/22) were *S. sonnei*, 32% (7/22) were *S. flexneri* and 9% (2/22) were *S. dysenteriae*1-7A. Pathogens were detected significantly more often in the patients than in the controls (44%, 85/193 vs 17%, 33/193); OR = 3.8; p < 0.0001).

For Kisumu District Hospital, the most common organisms isolated more often from cases as compared to the controls were rotavirus (10.5% vs 0.08%) and Shigella (8% vs 0.08%) and were significantly associated with diarrhea (OR = 10.2, OR = 13.9 respectively; p < 0.05) (Table 1). Other organisms isolated more frequently from the cases, though differences were not statistically significant, were C. jejuni, the diarrheagenic E. coli, Salmonella and C. parvum. G. lamblia and E. histolytica/E. dispar (0.08% v 5.4%; p < 0.05) were detected more often in the controls (Table 1B). Of the 22 Shigella isolates detected in the cases, 32% (6/19) were S. sonnei, 47% (9/19) were S. flexneri and 5% (1/19) were S. dysenteriae1-7A. Pathogens were detected significantly more often in the patients than in the controls (48%, 115/239 vs 18%, 43/239; OR = 3.5; p < 0.0001).

The prevalence of age-specific pathogens for Kericho and Kisumu District Hospitals is shown in Table 2. Rotavirus isolates and *G. lamblia* were found significantly more often in children aged <5 years than in the other age groups collected from both Kericho and Kisumu District Hospitals (p < 0.05). Interestingly, 8.2% and 11% of the *Shigella* isolates from Kericho and Kisumu District Hospitals were detected in adults ≥20 years of age. There were no significant differences in the age-specific prevalence of the organisms detected at either site.

The antibiotic resistance pattern of bacterial pathogens isolated from patients and controls from Kericho and Kisumu District Hospitals is shown in Table 3. The antibiotic resistance data for patients and controls were combined for each hospital, as bacteria isolated from patients and those isolated from controls showed no significant difference in resistance pattern. Resistance to ampicillin (42–100%), tetracycline (50–92%) and trimethoprim/sulfamethoxazole (83–100%) was found for all organisms except for *C. jejuni* isolated from patients and controls from Kericho District Hospital (Table 3). Resistance to ciprofloxacin was found in 42% of the EAEC and 8% of the *Shigella* isolates. Of the diarrheagenic *E. coli* isolates, five EAEC isolates and one ETEC isolate were identified as organisms producing extended-spectrum beta-lactamases (ESBLs) (data not shown).

Resistance to tetracycline (67–88%), ampicillin (52–100%) and trimethoprim/sulfamethoxazole (67–100%) was detected in all bacterial isolates except for *C. jejuni* from Kisumu District Hospital (Table 3). One of the 11 *C. jejuni* isolates was multidrug resistant to all of the antibiotics tested. Sensitivity to ciprofloxacin was found for all bacterial pathogens though there was resistance detected in the ETEC and EAEC isolates (27% and 50% respectively). Of the diarrheagenic *E. coli* isolates from

Table 1. Enteric pathogens in patients and controls from Kericho and Kisumu District Hospitals, Kenya

Kericho	Cases $(n = 193)$	Controls (n = 193)	OR (95% CI)	p value
Shigella	22 (11)	2 (1)	12.3 (2.8-53.1)	0.0001
Campylobacter	2 (1)	1 (0.5)	2.0 (0.1-22.14)	NS
EAEC	6 (3)	6 (3)	1.0 (0.3 – 3.1)	NS
EHEC	6 (3)	2 (1)	3.1 (0.6-15.4)	NS
ETEC	3 (1.5)	2 (1)	1.5 (0.2-9.1)	NS
Salmonella	1 (0.5)	1 (0.5)	1.0 (0.06-16.1)	NS
Rotavirus	20 (10.2)	6 (3)	3.6 (1.4-9.2)	0.007
Giardia lamblia	19 (10)	17 (9)	1.1 (0.5 – 2.4)	NS
Entamoeba histolytica/E. dispar	6 (3)	2 (1)	3.1 (0.6-15.4)	NS
Cryptospordium parvum	1 (0.5)	5 (2.6)	0.2 (0.01-1.7)	NS
Unidentified	107 (56)	159 (83)	3.8 (2.4-6.0)	0.0001
Kisumu	Cases $(n = 239)$	Controls ($n = 239$)	OR (95 CI)	p value
Shigella	19 (8)	2 (0.08)	10.2 (2.3-44.5)	0.0002
Campylobacter	5 (2.1)	4 (1.7)	1.2 (0.3 – 4.7)	NS
EAEC	6 (2.5)	2 (0.08)	3.1 (0.6-15.3)	NS
EHEC	2 (0.08)	1 (0.04)	2.0 (0.2-22.3)	NS
ETEC	11 (3.8)	4 (1.7)	2.8 (0.9-9.1)	NS
Salmonella	5 (2.1)	1 (0.04)	5.1 (0.6-43.9)	NS
Rotavirus	25 (10.5)	2 (0.08)	13.9 (3.3-59.4)	0.0001
Giardia lamblia	8 (2.7)	14 (6)	0.6 (0.2 – 1.4)	NS
Entamoeba histolytica/E. dispar	2 (0.08)	13 (5.4)	0.1 (0.03-0.7)	0.007
Cryptospordium parvum	2 (0.08)	1 (0.04)	2.0 (0.1-22.3)	NS
Unidentified	154 (52)	195 (82)	3.5 (2.3 – 5.4)	0.0001

Data are presented as no. (%).

OR and 95% confidence intervals calculated using multivariate logistic regression with a significant p value at < 0.05.

NS: not significant. EAEC: enteroaggregative Escherichia coli; ETEC: enterotoxigenic E. coli; EHEC: enterohemorrhagic E. coli.

Kisumu District Hospital, one EAEC isolate and three ETEC isolates were identified as ESBL-producing organisms (data not shown).

Discussion

Diarrhea can be caused by a plethora of microbial pathogens and it is not unusual in diarrheal disease surveillance studies to find that in a large percentage of patients the etiology is unknown. Furthermore, enteric infections including G. lamblia, Campylobacter and the diarrheagenic E. coli have been detected in asymptomatic individuals in numerous studies. It is therefore difficult to define what is causing the disease without directly comparing symptomatic patients and asymptomatic controls. 18-21 In the current case-control study, the predominant pathogens associated with patients with acute diarrhea as compared to the asymptomatic controls from both Kericho and Kisumu District Hospital were rotavirus and Shigella. The most common Shigella species were S. flexneri and S. sonnei. The bacterial pathogens identified in this study are consistent with those found in previous studies conducted in western Kenya, although none of these studies conducted surveillance in Kericho directly and none was a case-control study.^{8,22,23} In previous diarrhea casecontrol studies, ETEC, Shigella and rotavirus have been identified more frequently in symptomatic individuals than in controls.^{24–2}

Here, the diarrheagenic *E. coli* (ETEC, EAEC and EHEC) were also detected in more patients than controls but the differences were not significant. *G. lamblia* and *E. histolytica/E. dispar* were detected more often in the controls from Kisumu District Hospital than in the patients, and there was little difference in the number of EAEC, ETEC and *G. lamblia* isolates in patients and controls from Kericho District Hospital. Several studies have shown that asymptomatic individuals can have a high prevalence of *Giardia* and the diarrheagenic *E. coli* without displaying any overt symptoms.^{27, 28} Additionally, ova and parasite examination via microscopy and the Triage Parasite Panel are unable to distinguish between the pathogenic *E. histolytica* and non-pathogenic *E. dispar*. ^{16,29, 30} Controls from Kisumu District Hospital could have been harboring the non-pathogenic *E. dispar* as opposed to *E. histolytica*.

Rotavirus was found more often in individuals <5 years of age at both sites. These results are consistent with previous rotavirus surveillance studies conducted in Kenya.^{31, 32} The diarrheagenic *E.* coli, *G.* lamblia, *E.* histolytica/E. dispar and *C.* jejuni were found more often in children while *Shigella* was isolated more often from adults ≥ 20 years of age. Two previous studies conducted in western Kenya showed a similar trend, in which adults ≥ 20 years of age were infected more often with *Shigella*.^{8, 22}

Sources of water used by both patients and controls were ascertained from the questionnaires completed at the time of study enrollment. However, a disproportionate amount of the

Table 2. Enteric organisms from combined patients and controls from Kericho and Kisumu District Hospitals, Kenya, according to age group

Kericho	0-1 year (n = 148)	2-5 years (n = 147)	6-19 years (n = 36)	20+ years (n = 55)
Campylobacter	1 (0.7)	0 (0)	0 (0)	1 (2)
Shigella	5 (3.5)	8 (5.7)	1 (3.3)	4 (8.2)
Salmonella	0 (0)	2 (1.4)	0 (0)	0 (0)
EHEC	3 (2.1)	4 (2.8)	1 (3.3)	2 (4.1)
ETEC	2 (1.4)	2 (1.4)	0 (0)	1 (2)
EAEC	2 (1.4)	2 (1.4)	1 (3.3)	3 (6.1)
Rotavirus	15 (10.6)	4 (2.8)	2 (6.6)	0 (0)
G. lamblia	12 (8.5)	22 (15.6)	2 (6.6)	2 (4.1)
E. histolytica/E. dispar	0 (0)	6 (4.3)	0 (0)	1 (2)
C. parvum	0 (0)	4 (2.8)	0 (0)	1 (2)
Kisumu	0-1 year (n = 263)	2-5 years (n = 77)	6-19 years (n = 39)	20 + years (n = 153)
Campylobacter	4 (1.5)	4 (5.2)	0 (0)	1 (0.6)
Shigella	4 (1.5)	4 (5.2)	1 (2.6)	11 (7.2)
Salmonella	3 (1.1)	1 (1.3)	0 (0)	2 (1.3)
EHEC	1 (0.4)	0 (0)	2 (5.2)	0 (0)
ETEC	8 (3)	3 (3.9)	0 (0)	3 (2)
EAEC	6 (2.3)	0 (0)	0 (0)	2 (1.3)
Rotavirus	23 (8.7)	2 (2.6)	0 (0)	2 (1.3)
G. lamblia	12 (4.6)	8 (10.4)	0 (0)	2 (1.3)
E. histolytica/E. dispar	8 (3)	2 (2.6)	1 (2.6)	4 (2.6)
C. parvum	2 (0.8)	1 (1.3)	0 (0)	0 (0)

Data are presented as no. (%).

EHEC: enterohemorrhagic E. coli; ETEC: enterotoxiqenic E. coli; EAEC: enteroaggregative E. coli.

Table 3. Antibiotic resistance of enteric bacteria isolates from Kericho and Kisumu District Hospitals, Kenya

Kericho	Shigella (n $=$ 24)	Salmonella $(n = 2)$	ETEC $(n = 5)$	EAEC $(n = 12)$	EHEC $(n = 8)$
Ampicillin	10 (42)	2 (100)	4 (80)	11 (92)	5 (63)
TMP-SXT	20 (83)	2 (100)	5 (100)	10 (83)	8 (100)
Tetracycline	17 (71)	1 (50)	3 (60)	11 (92)	6 (75)
Ciprofloxacin	2 (8)	0 (0)	0 (0)	5 (42)	0 (0)
Kisumu	Shigella (n $=$ 21)	Salmonella $(n = 6)$	ETEC $(n = 15)$	EAEC $(n = 8)$	EHEC $(n = 3)$
Ampicillin	11 (52)	4 (67)	14 (93)	8 (100)	2 (67)
TMP-SXT	20 (95)	4 (67)	10 (67)	8 (100)	2 (67)
Tetracycline	15 (71)	4 (67)	11 (73)	7 (88)	2 (67)
Ciprofloxacin	0 (0)	0 (0)	4 (27)	4 (50)	0

Data are presented as no. (%).

TMP-SXT: trimethoprim/sulfamethoxazole); ETEC: enterotoxigenic E. coli; EAEC: enteroaggregative E. coli; EHEC: enterohemorrhagic E.coli.

patients and controls used municipal water so no significant correlations could be determined from this data. Several studies have examined the quality of water used in slums and rural areas and found significant levels of fecal coliforms and enteric viruses such as rotaviruses. However, no water quality studies to date have been conducted in Kisumu and Kericho directly. No information on travel to rural villages and homes and water used while there was captured in the questionnaire used in this study.

More than half of the *Shigella, Salmonella,* ETEC, EAEC and EHEC isolates were multidrug resistant to ampicillin, tetracycline and trimethoprim/sulfamethoxazole. These antibiotics are commonly prescribed and readily available at local chemists in Kisumu and Kericho. The emergence of multidrug resistant bacteria is an ongoing problem in Kenya, as a result of ready access to the drugs, unwarranted use of the drugs in clinical settings without proper laboratory diagnosis, and the ease of genetic exchange of resistance genes among the bacterial pathogens. ^{36,37}

WHO guidelines state that antibiotic use is warranted only in cases of shigellosis.³⁸ Empiric diagnosis of the cause of acute diarrhea at these medical facilities could result in unnecessary prescription of antibiotics.

Bacterial resistance to ampicillin, tetracycline and trimetho-prim/sulfamethoxazole has been well documented in Kenya and the results observed in this study are comparable to the antibiotic resistance observed in previous studies. 8,39-41 Most of the bacterial isolates were susceptible to ciprofloxacin; exceptions were several ETEC and EAEC isolates. These EAEC and ETEC isolates, classified by their antibiotic resistance pattern as extended-spectrum beta-lactamase producing organisms, were also resistant to aztreonam, levofloxacin, cefotaxime, ceftriax-one[CM1], imipenem and ceftazidine. Extended-spectrum beta-lactamase producing organisms are a major concern, with regard to treatment of diarrheal disease and other diseases of bacterial origin. 42,43 This emerging antibiotic resistance has been observed in bacterial pathogens in addition to EAEC and ETEC, including Salmonella and Klebsiella in Kenya. 44-46

The laboratory surveillance data observed in this study reflects only the population in Kisumu and Kericho who presented at the district hospitals for care and treatment and were not admitted to the respective hospitals. Therefore the data may underestimate the true burden of acute diarrheal disease in Kisumu and Kericho. The laboratory testing did not include testing for noroviruses, astroviruses and adenoviruses, which all have been found to be causes of acute diarrhea in Kenya, particularly in children. ^{47–49} Primers for enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC) were not included in the diarrheagenic *E. coli* PCR assay. As with the previously mentioned enteric viruses, these organisms have been found to cause acute diarrhea in Kenyan children. ^{12,50,51} These other organisms could have been responsible for the diarrhea in patients in which a pathogen was not detected.

The median age for all patients and controls from Kericho and Kisumu District Hospitals was less than 2 years, which reflects the greater burden of diarrheal illness in children than in the adult populations in Kisumu and Kericho. HIV status was not ascertained from the patients or controls from Kisumu and Kericho, so we were unable to compare enteric pathogens isolated from HIV-infected and uninfected individuals. A previous study conducted in Kisumu showed that HIV-infected children aged <2 years had higher rates of diarrhea when compared to HIV-uninfected children, though there were no significant correlations with any specific pathogen.⁵² The increase in *Shigella* isolates detected in adults ≥20 years of age in both Kericho and Kisumu could be attributable to the increased HIV prevalence in this age group and to these individuals seeking care more often because of their status.^{8,22,53} According to the Kenyan Demographic and Health Survey, 6.7% of Kenya's adult (15-49 years) population is infected with HIV, and HIV prevalence rates for the Rift Valley Province (Kericho) and Nyanza Province (Kisumu) are 5.3% and 15.1% respectively. 52,54

Future investigations should include establishing additional surveillance sites in the rural areas of Kericho and Kisumu, to include those individuals who do not have the means to be seen at the district hospitals. Additionally, HIV status of the individuals should be ascertained in future studies. And for future studies the enteric pathogens testing menu should be expanded to include norovirus, astrovirus, adenovirus, EPEC and EIEC. With

this data, intermediate diarrhea treatment strategies for outpatients at Kericho and Kisumu Dstrict hospital should adhere to the use of oral rehydration only, because antibiotics should be administered only in cases of known shigellosis. If antibiotics are to be prescribed, ciprofloxacin should be the drug of choice as most bacterial isolates were susceptible. Furthermore, education on proper hand washing and improved sanitation and hydiene should be emphasized to the patients.

Conclusion

The current study was a case-control study aimed at determining the etiology of acute diarrhea in an outpatient setting at two district hospitals in western Kenya. Shigella and rotavirus were isolated more often in the patients than in the controls from Kericho and Kisumu District Hospitals. Children <5 years old had the highest burden of acute diarrhea and most of the enteric pathogens detected were from this population. Significant numbers of asymptomatic controls, particularly those from Kisumu District Hospital, had higher numbers of G. lamblia, E. histolytica/E. dispar and C. parvum. Multidrug resistance to the most common antibiotics was evident in Kericho and Kisumu. Case-control studies of acute diarrhea using asymptomatic controls allow us to gain a much more accurate understanding of the disease burden and pathogen prevalence in a particular area. A more defined picture will allow for the development of community education programs and other interventions that could decrease the incidence of acute diarrhea.

Authors' contributions: EVO, MTW, CZ and JEL conceived the study and designed the study protocol. BES was the primary investigator. EAO, CPO, BES, EKC, RKK and JNN conducted the laboratory analysis. BES, MCK, EAO, CPO, EKC, RKK and JNN analysed the data. BES and EVO wrote the manuscript. All authors reviewed the manuscript, improved the content and approved the final version. BES is guarantor of the paper.

Acknowledgements: The authors thank the following individuals: Duke Omariba, Margaret Bii, Caroline Tegerei, Alice Sang, Catherine Muriuk and Eunice Owiti for their hard work and dedication in facilitating the study at Kericho and Kisumu District Hospitals; Douglas Shaffer, Scott Gordon, Kent Kester, Robert Bowden and David Schnabel for their establishment and unwavering support of the microbiology laboratory in Kericho; Chad Porter for reviewing the manuscript; the Director of the Kenya Medical Research Institute for his support; and Thomas Logan for his continued support and review of the manuscript.

Funding: This study was funded by the Armed Forces Health Surveillance Center-Global Emerging Infections Surveillance and Response Systems, Silver Spring, MD, USA.

Conflicts of Interest: None declared.

Ethical approval: This study was approved by the Kenya Medical Research Institute Ethical Review Committee and the Walter Reed Army Institute of Research Institutional Review Board.

References

1 Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997;349:1269-76.

- 2 Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. Bull World Health Organ 2008; 86:710-7.
- 3 Ramani S, Kang G. Viruses causing childhood diarrhoea in the developing world. Curr Opin Infect Dis 2009;22:477–82.
- 4 Karanis P, Kourenti C, Smith H. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health 2007;5:1–38.
- 5 Guerrant RL, Hughes JM, Lima NL, Crane J. Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. Rev Infect Dis 1990;12(Suppl 1):S41–50.
- 6 Jiang ZD, Lowe B, Verenkar MP et al. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). J Infect Dis 2002;185:497–502.
- 7 Mugoya I, Kariuki S, Galgalo T et al. Rapid spread of *Vibrio cholerae* O1 throughout Kenya, 2005. Am J Trop Med Hyg 2008;78:527–33.
- 8 Brooks JT, Ochieng JB, Kumar L et al. Surveillance for bacterial diarrhea and antimicrobial resistance in rural western Kenya, 1997–2003. Clin Infect Dis 2006;43:393–401.
- 9 Chunge RN, Nagelkerke N, Karumba PN et al. Longitudinal study of young children in Kenya: intestinal parasitic infection with special reference to *Giardia lamblia*, its prevalence, incidence and duration, and its association with diarrhoea and with other parasites. Acta Trop 1991;50:39–49.
- 10 Nyarango RM, Aloo PA, Kabiru EW, Nyanchongi BO. The risk of pathogenic intestinal parasite infections in Kisii Municipality, Kenya. BMC Public Health 2008;8:237.
- 11 Gatei W, Ashford RW, Beeching NJ et al. *Cryptosporidium muris* infection in an HIV-infected adult, Kenya. Emerg Infect Dis 2002; 8:204–6
- 12 Saidi SM, Ijjima Y, Sang WK et al. Epidemiological study on infectious diarrheal diseases in children in a coastal rural area of Kenya. Microbiol Immunol 1997;41:773–8.
- 13 Joyce T, McGuigan KG, Elmore-Meegan M, Conroy RM. Prevalence of enteropathogens in stools of rural Maasai children under five years of age in the Maasailand region of the Kenyan Rift Valley. East Afr Med J 1996;73:59–62.
- 14 Davis L, DiRita V. Growth and laboratory maintenance of *Campylobacter jejuni*. Curr Protoc Microbiol 2008; Chapter 8: Unit 8A.1.1–8A.1.7.
- 15 Grimes KA, Mohamed JA, Dupont HL et al. PCR-based assay using occult blood detection cards for detection of diarrheagenic *Escherichia coli* in specimens from U.S. travelers to Mexico with acute diarrhea. J Clin Microbiol 2008;46:2227–30.
- 16 Swierczewski B, Odundo E, Ndonye J et al. Comparison of the triage micro parasite panel and microscopy for the detection of Entamoeba histolytica/Entamoeba dispar, Giardia lamblia, and Cryptosporidium parvum in stool samples collected in Kenya. J Trop Med 2012; doi: 10.1155/2012/564721.
- 17 Garcia LS. Diagnostic medical parasitology. 4th ed. Washington, DC: American Society for Microbiology; 2001.
- 18 Ali MB, Ghenghesh KS, Aissa RB et al. Etiology of childhood diarrhea in Zliten, Libya. Saudi Med J 2005;26:1759–65.
- 19 Baqui AH, Sack RB, Black RE et al. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children less than 5 years of age. J Infect Dis 1992;166:792–6.
- 20 El-Mohamady H, Abdel-Messih IA, Youssef FG et al. Enteric pathogens associated with diarrhea in children in Fayoum, Egypt. Diagn Microbiol Infect Dis 2006;56:1–5.

- 21 Schorling JB, Wanke CA, Schorling SK. A prospective study of persistent diarrhea among children in an urban Brazilian slum. Patterns of occurrence and etiologic agents. Am J Epidemiol 1990;132:144–56.
- 22 Brooks JT, Shapiro RL, Kumar L et al. Epidemiology of sporadic bloody diarrhea in rural Western Kenya. Am J Trop Med Hyg 2003;68:671–7.
- 23 Malakooti MA, Alaii J, Shanks GD, Phillips-Howard PA. Epidemic dysentery in western Kenya. Trans R Soc Trop Med Hyg 1997; 91:541–3.
- 24 Bodhidatta L, McDaniel P, Sornsakrin S, Srijan A, Serichantalergs O, Mason CJ. Case-control study of diarrheal disease etiology in a remote rural area in Western Thailand. Am J Trop Med Hyg 2010; 83:1106-9
- 25 Albert MJ, Faruque AS, Faruque SM, Sack RB, Mahalanabis D. Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. J Clin Microbiol 1999;37:3458–64.
- 26 Vu Nguyen T, Le Van P, Le Huy C et al. Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam. Int J Infect Dis 2006;10:298–308.
- 27 Hien BT, Trang do T, Scheutz F et al. Diarrhoeagenic *Escherichia coli* and other causes of childhood diarrhoea: a case-control study in children living in a wastewater-use area in Hanoi, Vietnam. J Med Microbiol 2007;56(Pt. 8):1086–96.
- 28 Gilman RH, Marquis GS, Miranda E et al. Rapid reinfection by *Giardia lamblia* after treatment in a hyperendemic Third World community. Lancet 1988;1:343–5.
- 29 Diamond LS, Clark CG. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. J Eukaryot Microbiol 1993;40: 340–4.
- 30 Garcia LS, Shimizu RY, Bernard CN. Detection of *Giardia lamblia*, Entamoeba histolytica/Entamoeba dispar, and Cryptosporidium parvum antigens in human fecal specimens using the triage parasite panel enzyme immunoassay. J Clin Microbiol 2000;38: 3337–40.
- 31 Kiulia NM, Kamenwa R, Irimu G et al. The epidemiology of human rotavirus associated with diarrhoea in Kenyan children: a review. J Trop Pediatr 2008;54:401–5.
- 32 Nyangao J, Page N, Esona M et al. Characterization of human rotavirus strains from children with diarrhea in Nairobi and Kisumu, Kenya, between 2000 and 2002. J Infect Dis 2010;202(Suppl): S187–92.
- 33 Kiulia NM, Netshikweta R, Page NA et al. The detection of enteric viruses in selected urban and rural river water and sewage in Kenya, with special reference to rotaviruses. J Appl Microbiol 2010;109:818–28.
- 34 Onyango-Ouma W, Gerba CP. Away-from-home drinking water consumption practices and the microbiological quality of water consumed in rural western Kenya. J Water Health 2011;9:628–36.
- 35 Kimani-Murage EW, Ngindu AM. Quality of water the slum dwellers use: the case of a Kenyan slum. J Urban Health 2007;84:829–38.
- 36 O'Brien TF. The global epidemic nature of antimicrobial resistance and the need to monitor and manage it locally. Clin Infect Dis 1997;24(Suppl 1):S2-8.
- 37 Finch MJ, Morris JG Jr, Kaviti J et al. Epidemiology of antimicrobial resistant cholera in Kenya and East Africa. Am J Trop Med Hyg 1988;39:484–90.
- 38 WHO. The treatment of diarrhoea: A manual for physicians and other senior health workers. Geneva: World Health Organization; 2005. http://

- www.who.int/maternal_child_adolescent/documents/9241593180/en/ [accessed 27 August 2012].
- 39 Oundo JO, Kariuki S, Maghenda JK, Lowe BS. Antibiotic susceptibility and genotypes of non-typhi *Salmonella* isolates from children in Kilifi on the Kenya coast. Trans R Soc Trop Med Hyg 2000;94:212–5.
- 40 Shapiro RL, Kumar L, Phillips-Howard P et al. Antimicrobial-resistant bacterial diarrhea in rural western Kenya. J Infect Dis 2001;183: 1701–4.
- 41 Oundo JO, Kariuki SM, Boga HI et al. High incidence of enteroaggregative *Escherichia coli* among food handlers in three areas of Kenya: a possible transmission route of travelers' diarrhea. J Travel Med 2008;15:31–8.
- 42 Kariuki S, Revathi G, Corkill J et al. *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. J Infect Dev Ctries 2007; 1:257–62.
- 43 Zahar JR, Lortholary O, Martin C et al. Addressing the challenge of extended-spectrum beta-lactamases. Curr Opin Investig Drugs 2009;10:172–80.
- 44 Kiiru J, Kariuki S, Goddeeris BM et al. *Escherichia coli* strains from Kenyan patients carrying conjugatively transferable broad-spectrum beta-lactamase, qnr, aac(6')-Ib-cr and 16S rRNA methyltransferase genes. J Antimicrob Chemother 2011;66:1639–42.
- 45 Boyle F, Healy G, Hale J et al. Characterization of a novel extendedspectrum beta-lactamase phenotype from OXA-1 expression in Salmonella typhimurium strains from Africa and Ireland. Diagn Microbiol Infect Dis 2011;70:549–53.

- 46 Poirel L, Revathi G, Bernabeu S, Nordmann P. Detection of NDM-1-producing *Klebsiella pneumoniae* in Kenya. Antimicrob Agents Chemother 2011; 55:934–6.
- 47 Nakata S, Honma S, Numata K et al. Prevalence of human calicivirus infections in Kenya as determined by enzyme immunoassays for three genogroups of the virus. J Clin Microbiol 1998;36:3160–3.
- 48 Magwalivha M, Wolfaardt M, Kiulia NM et al. High prevalence of species D human adenoviruses in fecal specimens from Urban Kenyan children with diarrhea. J Med Virol 2010;82:77–84.
- 49 Kiulia NM, Mwenda JM, Nyachieo A et al. Astrovirus infection in young Kenyan children with diarrhoea. J Trop Pediatr 2007;53:206–9.
- 50 Sang WK, Boga HI, Waiyaki PG et al. Prevalence and genetic characteristics of Shigatoxigenic *Escherichia coli* from patients with diarrhoea in Maasailand, Kenya. *J* Infect Dev Ctries 2012;6:102–8.
- 51 O'Reilly CE, Jaron P, Ochieng B, Nyaguara A, Tate JE, Parsons MB et al. Risk factors for death among children less than 5 years old hospitalized with diarrhea in rural western Kenya, 2005–2007: A Cohort Study. PLoS Med 2012;9:pe1001256.
- 52 van Eijk AM, Brooks JT, Adcock PM et al. Diarrhea in children less than two years of age with known HIV status in Kisumu, Kenya. Int J Infect Dis 2010;14:e220-5.
- 53 Davies NE, Karstaedt AS. *Shigella* bacteraemia over a decade in Soweto, South Africa. Trans R Soc Trop Med Hyg 2008;102:1269–73.
- 54 Foglia G, Sateren WB, Renzullo PO et al. High prevalence of HIV infection among rural tea plantation residents in Kericho, Kenya. Epidemiol Infect 2008;136:694–702.